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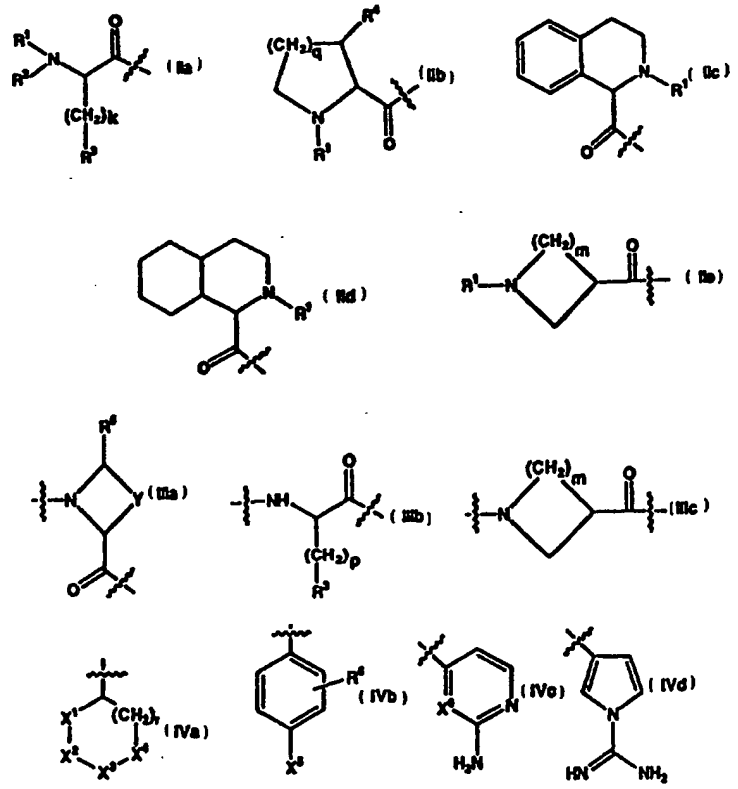
(54) Title: NEW PEPTIDE DERIVATIVES

(57) Abstract

The invention relates to new competitive inhibitors of trypsin-like serine proteases, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds as thrombin inhibitors, anticoagulants and anti-inflammatory inhibitors for prophylaxis and treatment of related diseases, according to the formulas (I): A^1-A^2-NH-$(CH_2)_n$-B and (V): A^1-A^2-NH-$(CH_2)_n$-B-D wherein A^1 represents a structural fragment of formulas (IIa), (IIb), (IIc), (IId), (IIe), A^2 represents a structural fragment of formulas (IIIa), (IIIb), (IIIc), B represents a structural fragment of formulas (IVa), (IVb), (IVc), (IVd). Further described are novel compounds, the new use of compounds and especially new structural fragment in synthesis of pharmaceutical compounds.



New peptid d rivativ s

This invention relates to new competitive inhibitors of trypsin-like serine proteases, especially thrombin and kininogenases such as kallikrein, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds as thrombin inhibitors and anticoagulants and as antiinflammatory inhibitors, respectively.
The invention also relates to novel use of compounds as starting materials in synthesis of a serine protease inhibitor. Furthermore the invention relates to a novel structural fragments in serine protease inhibitors.

BACKGROUND

Blood coagulation is the key process involved in both haemostasis (i.e. prevention of blood loss from a damaged vessel) and thrombosis (i.e. the pathological occlusion of a blood vessel by a blood clot). Coagulation is the result of a complex series of enzymatic reactions, where one of the final steps is conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin plays a central role in coagulation. It activates platelets, it converts fibrinogen into fibrin monomers, which polymerise spontaneously into filaments, and it activates factor XIII, which in turn crosslinks the polymer to insoluble fibrin. Thrombin further activates factor V and factor VIII in a positive feedback reaction. Inhibitors of thrombin are therefore expected to be effective anticoagulants by inhibition of platelets, fibrin formation and fibrin stabilization. By inhibiting the positive feedback

mechanism they are expected to excert inhibition early in the chain of events leading to coagulation and thrombosis.

Kininogenases are serine proteases that act on kininogens to produce kinins (bradykinin, kallidin, and Met-Lys-bradykinin). Plasma kallikrein, tissue kallikrein, and mast cell tryptase represent important kininogenases.

Kinins (bradykinin, kallidin) are generally involved in inflammation. For example, the active inflammation process is associated with increased permeability of the blood vessels resulting in extravasation of plasma into the tissue. The ensuing plasma exudate contains all the protein systems of circulating blood. The plasma-derived kininogens inevitably will be interacting with different kallikreins, forming kinins continually as long as the active plasma exudation process is ongoing. Plasma exudation occurs independent of the mechanisms that are involved in the inflammation, whether it is allergy, infection or other factors (Persson et al., Editorial, Thorax, 1992, 47:993-1000). Plasma exudation is thus a feature of many diseases including asthma, rhinitis, common cold, and inflammatory bowel diseases. Particulary in allergy mast cell tryptase will be released (Salomonsson et al., Am. Rev. Respir. Dis., 1992, 146:1535-1542) to contribute to kinin formation and other pathogenic events in asthma, rhinitis, and intestinal diseases.

The kinins are biologically highly active substances with smooth muscle effects, sectretory effects, neurogenic effects, and actions that may perpetuate inflammatory processes including activation of phospholipas A_2 and incr asing vascular permeability. The latter action pot ntially induces a vicious circle

with kinins providing for the generation of m re kinins etc.

Tissue kallikrein cleaves primarily low molecular weight kininogen to produce kallidin and plasma kallikrein preferably releases bradykinin from high molecular weight kininogen.

PRIOR ART

Inhibitors of thrombin based on the amino acid sequence around the cleavage site for the fibrinogen Aα chain were first reported by Blombäck et al. in J. Clin. Lab. Invest. 24, suppl 107, 59, (1969), who suggested the sequence Phe-Val-Arg (P9-P2-P1, herein referred to as the P3-P2-P1 sequence) to be the best inhibitor.

In US 4,346,078 has S. Bajusz et al. described the thrombin inhibitor H-DPhe-Pro-Agm, a dipeptidyl derivative with an aminoalkyl guanidine in the P1-position.

Inhibitors of thrombin based on peptide derivatives with a cyclic aminoalkyl guanidine, e.g. 3-aminomethyl-1-amidinopiperidine, in the P1-position have been disclosed in EP-A2-0,468,231.

In EP-A2-0,185,390 has S. Bajusz et. al. disclosed that replacing the agmatine with an arginine aldehyde gave a thrombin inhibitor which had much higher potency.

Inhibitors of kallikrein based on the amino acid sequence around the cleavage site Arg-Ser have been reported earlier.

The arginine chloromethyl ketones H-DPro-Ph -Arg-CH_2Cl

and H-D Phe-Phe-Arg-CH_2Cl wer reported as plasma kallikrein inhibitors by Kettn r and Shaw in Biochemistry 1978, 17:4778-4784 and Meth. Enzym. 1981, 80:826-842.

Likewise, esters and amides containing the H-DPro-Phe-Arg sequence were reported by Fareed et al. in Ann. N.Y. Acad. Sci. 1981, 370:765-784 to be plasma kallikrein inhibitors.

Inhibitors of serine proteases that are based on electrophilic ketones instead of aldehydes in the P1-position are described in the following patent documents:

EP-A2-0,195,212 describing peptidyl α-keto esters and amides, EP-A1-0,362,002 describing fluoroalkylamide ketones and EP-A2-0,364,344 describing α,β,δ- triketo compounds possessing different peptidase inhibiting properties.

Inhibitors of trypsin-like serine proteases, such as thrombin and kallikrein, based on C-terminal boronic acid derivatives of arginine and isothiouronium analogues thereof have been revealed in EP-A2-0,293,881.

WO 92/04371 describing kininogenase inhibitors, e.g. kallikrein inhibitors based on derivatives of arginine.

EP-A1-0,530,167 describing α-alkoxy ketone derivatives of arginine as thrombin inhibitors.

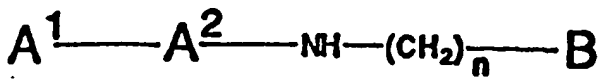
DISCLOSURE OF THE INVENTION

An object f the present invention is to provide novel

and potent trypsine-like s rine protease inhibitors, especially anticoagulantia and antiinflammatory compounds with competitive inhibitory activity towards their enzyme i.e. causing reversible inhibition. More specifically anticoagulants for prophylaxis and treatment of thromboembolic diseases such as venous thrombosis, pulmonary embolism, arterial thrombosis, in particular myocardial infarction and cerebral thrombosis, general hypercoagulable states and local hypercoagulable states, e.g. following angioplasty and coronary bypass operations, and other situations where thrombin is believed to play a role, e.g. Alzheimers disease, as well as inhibition of kininogenases for treatment of inflammatory disorders e.g. asthma, rhinitis, urticaria, inflammatory bowel disease, and arthritis. A further object is to obtain thrombin inhibitors which are orally bioavailable and selective in inhibiting thrombin over other serine proteases. A further object of the invention is to obtain kininogenase inhibitors which can be given orally, rectally, topically e.g. dermally, or via the inhalation route.

Compounds

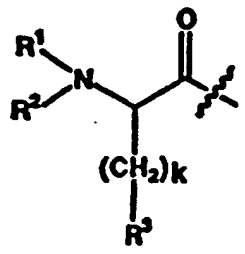
According to the invention it has been found that compounds of the general Formula I, either as such or in the form of physiologically acceptable salts, and including stereoisomers, are potent inhibitors of serine proteases, especially thrombin and kininogenases such as kallikrein:



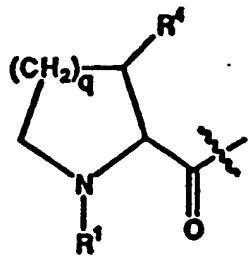
Formula I

wherein:

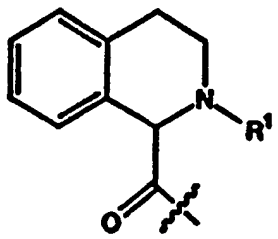
A^1 represents a structural fragment of Formula IIa, IIb, IIc, IId or IIe;



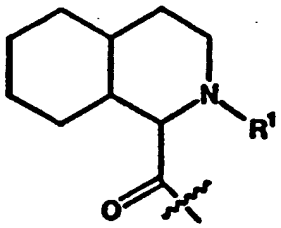
IIa



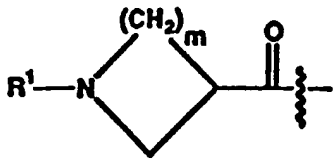
IIb



IIc



IId



IIe

wherein:

k is an integer 0, 1, 2, 3 or 4;

m is an integer 1, 2, 3 or 4;

q is an integer 0, 1, 2 or 3;

R^1 represents H, an alkyl group having 1 to 4 carbon atoms, or $R^{11}OOC$-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted in the position which is alpha to the carbonyl group, and the alpha substituent is a group $R^{17}-(CH_2)_p-$, wherein p is

0,1 or 2 and R^{17} is m thyl, phenyl, OH, $COOR^{12}$, $CONHR^{12}$, where R^{12} is H or an alkyl group having 1 t 4 carbon atoms, and R^{11} is H or an alkyl group having 1 to 6 carbon atoms, or

R^1 represents $Ph(4\text{-}COOR^{12})\text{-}CH_2\text{-}$, where R^{12} is as defined above, or

R^1 represents $R^{13}\text{-}NH\text{-}CO\text{-}alkyl\text{-}$, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R^{13} is H or an alkyl group having 1 to 4 carbon atoms or $-CH_2COOR^{12}$, where R^{12} is as defined above, or

R^1 represents $R^{12}OOC\text{-}CH_2\text{-}OOC\text{-}alkyl\text{-}$, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R^{12} is as defined above, or

R^1 represents $R^{14}SO_2\text{-}$, $Ph(4\text{-}COOR^{12})\text{-}SO_2\text{-}$, $Ph(3\text{-}COOR^{12})\text{-}SO_2\text{-}$, $Ph(2\text{-}COOR^{12})\text{-}SO_2\text{-}$, where R^{12} is as defined above and R^{14} is an alkyl group having 1-4 carbon atoms, or

R^1 represents $-CO\text{-}R^{15}$, wherein R^{15} is an alkyl group having 1-4 carbon atoms, or

R^1 represents $-CO\text{-}OR^{15}$, where R^{15} is as defined above, or

R^1 represent $-CO\text{-}(CH_2)_p\text{-}COOR^{12}$, where R^{12} is as defined above and p is an interger 0, 1 or 2, or

R^1 represents $-CH_2PO(OR^{16})_2$, $-CH_2SO_3H$ or $-CH_2\text{-}(5\text{-}(1H)\text{-}tetrazolyl)$, where R^{16} is, individually at each occurrence, H, methyl or ethyl;

R^2 represents H or an alkyl gr up having 1 to 4 carbon atoms or $R^{21}OOC$-alkyl-, where th alkyl gr up has 1 to 4 carbon atoms and, where R^{21} is H or an alkyl group having 1 to 4 carbon atoms;

R^3 represents an alkyl group having 1-4 carbon atoms, and the alkyl group may or may not carry one or more flourine atoms, or

R^3 represents a cyclopentyl, cyclohexyl- or a phenyl group which may or may not be substituted with an alkyl group having 1 to 4 carbon atoms, or

R^3 represents a phenyl group substituted with a OR^{31} group, where R^{31} is H or an alkyl group having 1 to 4 carbon atoms and k is 0, 1, or

R^3 represents a 1-naphthyl or 2-naphthyl group and k is 0, 1, or

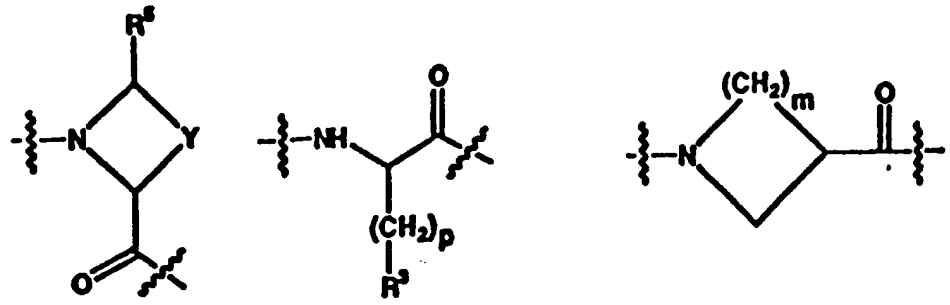
R^3 represent a cis- or trans-decalin group and k is 0, 1, or

R^3 represents 4-pyridyl, 3-pyrrolidyl or 3-indolyl which may or may not be substituted with a OR^{31} group, where R^{31} is as defined above and k is 0, 1, or

R^3 represents $Si(Me)_3$ or $CH(R^{32})_2$, wherein R^{32} is a cyclohexyl- or a phenyl group;

R^4 represents H, an alkyl group having 1 to 4 carbon atoms, a cyclohexyl- or a phenyl group;

A^2 represents a structural fragment of Formula IIIa, IIIb or IIIc



IIIa IIIb IIIc

wherein:

p is an interger 0, 1 or 2;

m is an integer 1, 2, 3 or 4;

Y represents a methylene group, or

Y represents an ethylene group and the resulting 5-membered ring may or may not carry one or two fluorine atoms, a hydroxy group or an oxo group in position 4, or may or may not be unsaturated, or

Y represents $-CH_2-O-$, $-CH_2-S-$, $-CH_2-SO-$, with the heteroatom functionality in position 4, or

Y represents a n-propylene group and the resulting 6-membered ring may or may not carry in position 5 one fluorine atom, a hydroxy group or an oxo group, carry two fluorine atoms in one of positions 4 or 5 or be unsaturated in position 4 and 5, or carry in position 4 an alkyl group with 1 to 4 carbon atoms, or

Y represents $-CH_2-O-CH_2-$, $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, or

Y represent $-CH_2-CH_2-CH_2-CH_2-$;

R^3 is as defined above;

(3RS)-1-(N,N'-di(benzyloxycarbonyl)amidino)-3-aminomethyl pyrrolidine (H-Nig(Z)$_2$),
(3RS)-1-(N-benzyloxycarbonylamidino)-3-aminoethyl pyrrolidine (H-Hig(Z)),
(3RS)-1-(N,N'-di(benzyloxycarbonyl)amidino)-3-aminoethyl pyrrolidine (H-Hig(Z)$_2$),
3-aminomethyl-1-(N-benzyloxycarbonylamidino) azetidine (H-Mig(Z)),
3-aminomethyl-1-(N,N'-di(benzyloxycarbonyl)amidino) azetidine (H-Mig(Z)$_2$),
3-aminoethyl-1-(N-benzyloxycarbonylamidino) azetidine (H-Dig(Z)),
3-aminoethyl-1-(N,N'-di(benzyloxycarbonyl)amidino) azetidine (H-Dig(Z)$_2$),

Said compounds are used as starting materials in the preparation of the claimed peptide derivatives of formulas I, Ia, Ib, V, Va and Vb.

Medical and pharmaceutical use

The invention also provides compositions and methods for the treatment, in a human or animal organism, of conditions where inhibition of thrombin is required and of physiologically disorders especially inflammatory diseases.

The thrombin inhibiting compounds of the invention are expected to be useful in particular in animals including man in treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues. They are furthermore expected to be useful in situations where there is an undesirable excess of the thrombin without signs of hypercoagulability, for example as in Alzheimers disease and pancreatitis. Disease states in which these compounds hav a potential utility, in treatment and/or prophylaxis, include ven us thrombosis

and pulmonary embolism, arterial thr mbosis, such as in myocardial infarction, unstable angina, thrombosis-based stroke and p ripheral arterial thr mbosis and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricule after transmural myocardial infarction. Further, these compounds have expected utility in prophylaxis of atherosclerotic diseases such as coronary arterial disease, cerebral arterial disease and peripheral arterial disease. Further, these compounds are expected to have synergistic antithrombotic effects when combined with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agent acetylsalicylic acid. Further, these compounds are expected to be useful together with thrombolytics in thrombotic diseases, in particular myocardial infarction. Further, these compounds have expected utility in prophylaxis for re-occlusion after thrombolysis, percutaneous trans-luminal angioplasty (PTCA) and coronary bypass operations. Further, these compounds have expected utility in prevention of re-thrombosis after microsurgery and vascular surgery in general. Further, these compounds have expected utility in treatment and prophylaxis of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism. Further, these compounds are expected to be useful in anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vasculars stemts, vascular catheters, mechanical and biological prosthetic or any other medical device. Further, these compounds have expected utility in anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using or heart-lung machine or in haemodialysis.

A further expected utility of the anticoagulant compounds of the invention ar in rinsing of catheters and mechanical devises used in patients in vivo, and as anticoagulants for preservation of blood, plasma and other blood products in vitro.

The antiinflammatory inhibiting compounds of the invention are expected to be useful in particular in animals including man in treatment or prophylaxis of inflammatory diseases such as asthma, rhinitis, pancreatitis, uticaria, inflammatory bowel diseases, and arthritis. An effective amount of kininogenase inhibiting compounds with or without a physiologically acceptable carrier or diluent can be used solely or in combination with other therapeutic agents.

The compounds inhibit the activity of kallikreins assessed with chromogenic substrates according to known procedures. The anti-inflammatory actions of the present compounds can for example be studied by their inhibition of allergen-induced exudative inflammatory processes in airway mucosa or gut mucosa.

Pharmaceutical preparations

The compounds of the invention will normally be administered orally, rectally, dermally, nasally, tracheally, bronchially, parenterally or via inhalation route, in the form of pharmaceutical preparations comprising the active ingredient either as a free base or a pharmaceutical acceptable non-toxic organic or inorganic acid addition salt, e.g. the hydrochloride, hydrobromide, sulphate, hydrosulphate, nitrate, lactate, acetate, citrate, bensoate, succinate, tartrate, trifluoroacetate and the like in a pharmac utically acceptable dosage form. Depending upon

27. A pharmaceutical pr paration comprising an effective amount of a compound as outlined in any of claims 1-4, 6-10 or 18-19 in conjuction with one or more pharmaceutical carriers for use as an antiinflammatory agent.

28. Use of compound according to any of claims 1-5 or 7-17 as an active ingredient for manufacture of a pharmaceutical preparation for inhibition of thrombin in a human or animal organism.

29. Use of compound according to any of claims 1-4, 6-10 or 18-19 as an active ingredient for manufacture of a pharmaceutical preparation for inhibition of kininogenases in a human or animal organism.

30. A method for obtaining inhibition of thrombin in a human or animal organism in need of such inhibition, comprising administering to said organism an inhibitory effective amount of a compound claimed in any of claims 1-5 or 7-17.

31. A method for obtaining inhibition of kininogenases in a human or animal organism in need of such inhibition, comprising administering to said organism an inhibitory effective amount of a compound claimed in any of claims 1-4, 6-10 or 18-19.

32. Use of a compound of the formula: